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Full Length Research Paper

Synthesis, characterization and trypanocidal activity of some aromatic thiosemicarbazones and their 1,3,4-thiadiazolines derivatives

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The thiosemicarbazones of six aromatic ketones were synthesized as well as their 1,3,4-thiadiazolines derivatives obtained by cyclization under acetylating condition with yields going from 40 to 90%. The products purity was confirmed by mass spectrometry coupled with high-performance liquid chromatography (LC/MS) and there were characterized using spectrometry IR, NMR ^{1}H and ^{13}C (nuclear magnetic resonance). These compounds were then tested *in vitro* on *Trypanosoma brucei brucei* according to the "LILIT, Alamar Blue" method for a comparison of their trypanocidal activity. Thus, all thiosemicarbazones appeared much more active than their corresponding 1,3,4-thiadiazolines. Thiosemicarbazone 6a (IC_{50} = 9.62 µM) was the most active of all thiosemicarbazones tested and it is the same for its thiadiazoline 6b (IC_{50} = 49.03 µM) among 1,3,4-thiadiazolines.

Key words: Thiosemicarbazone, 1,3,4-thiadiazolines, characterization, trypanocidal activity.

INTRODUCTION

African trypanosomes are parasitic protozoa that afflict both man and animals. *Trypanosoma brucei brucei* is one of the causative agents of "Nagana" which decimate cattle. *Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense* are responsible for the African human trypanosomiase, an endemic disease in sub-Saharan Africa with nearly 50.000 estimated cases and a population at risk of 60 million people (WHO 2007a). The toxicity and adverse effects of drugs that have been commonly used to treat this disease, their impractical dosing regimens (WHO, 2007b) as well as the damage caused in the sector of the breeding require the development of new active molecules in a most safe chemotherapeutic approach. Accordingly, our interest has been focused on the thiosemicarbazones which showed during the 50 last years a broad spectrum of therapeutic properties. Indeed, these small molecules show antiviral (García et al., 2004), antibacterial (San et al., 2003; Rebolledo et al., 2003; Kasuga et al., 2003), antitumor (Afrasiabi et al., 2004) anticonvulsant (Pendeya et al., 1999) antimalarial (Klayman et al., 1984), and antitrypanosomal activities (Doron et al., 2004)

In the research of new therapeutic tools for the treatment of trypanosomiasis, selected thiosemicarbazone compounds exhibit potent activity against cruzain and *T. brucei* cathepsin B as well as trypanocidal activity against parasites in cell culture. The non-peptidic nature of these compounds, coupled with their low cost of synthesis, makes this class of reversible covalent inhibitors very promising candidates for the development of new antitrypanosomal chemotherapy. Du et al. (2002) found some thiosemicarbazones inhibitors of the

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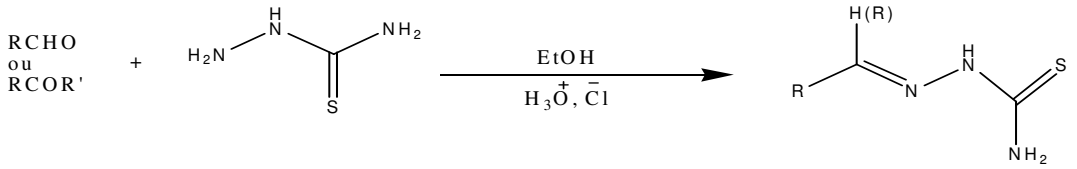


Figure 1. Synthesis of thiosemicarbazones.

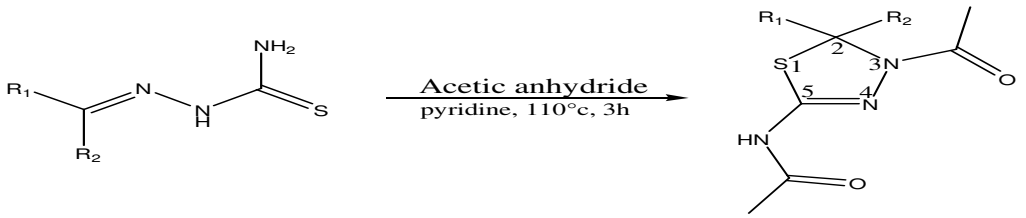


Figure 2. Synthesis of 1,3,4-thiadiazolines.

Trypanosoma cruzi cysteine protease "cruzain" The structure-activity relationship (SAR) indicates that aryl thiosemicarbzones is a productive scaffold for killing the parasites. More recently in 2003, Rebolledo et al. (2003) have synthesized 4-substituted nitroacetophenone thiosemicarbazones and their complexes with copper. These thiosemicarbazones tested for their growth inhibition of the epimastigote form of Trypanosoma cruzi, have shown comparable efficacy to that of reference compounds (nifurtimox and benznidazole) that have been commonly used in the treatment of trypanosomiasis.

The 1,3,4-thiadiazoles and 1,3,4-thiadiazolines which are cyclic derivatives of the thiosemicarbazones exhibit various biological activities such as antituberculosis antimicrobial (Mamolo et al., 1996) anti-inflammatory (Labanauskas et al., 2001) antiviral, anticonvulsant (Chapleo et al., 1988) antihypertensive (Mazzone et al., 1993) anticancer (Chou et al., 2003) and hypoglycemic activities (Hanna et al., 1993). Therefore, 1,3,4-Thiadiazole and related compounds are of great interest in chemistry owing to their bioactivity of certain plant growth regulating effects as well as antimicrobial activity (Sancak et al., 2007).

The thiosemicarbazones and 1,3,4-thiadiazolines thus presented have about the same biological properties however there is little information about the trypanocidal activity of 1,3,4-thiadiazolines. The aim of this work is to synthesize thiosemicarbazones and their corresponding 1,3,4-thiadiazolines in order to compare their trypanocidal activities.

EXPERIMENTALS

Chemistry

We used thin layer chromatography (TLC) to estimate the purity of the compounds, to follow the evolution of the reaction and then to achieve their purification on silica gel column. The solvent used is the mixture of dichloromethane/ethylacetate (2/1) or dichloromethane/methanol (9/1). The thiosemicarbazones were purified by recrystallization. Compounds purity was confirmed by LC/MS. The melting points were taken on the fusionometer *eletrothermal 1A 9000*. The spectrometric data were recorded with the following instruments: IR, Perkin Elmer FT-IR 286; ^{1}H NMR and ^{13}C NMR, Bruker 400; LC/MS in mode APCI on column C_{18}.

The thiosemicarbazones and 1,3,4-thiadiazolines are synthesized as follows:

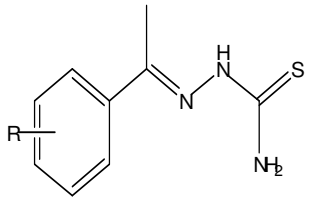
1) Synthesis of the thiosemicarbazones. A mixture of ketone (20 mmol dissolved in 100 m of ethanol) and thiosemicarbazide (20 mmol dissolved in 20 ml of 1 N hydrochloric acid) is stirred until the thiosemicarbazone precipitates. The precipitate is filtered, dried and then recrystallized in ethanol (96°C) to give thiosemicarbazone crystals (Figure 1).

2) Synthesis of 1,3,4-thiadiazolines. Thiosemicarbazone (0.25 mmol) was dissolved in 0.5 ml of pyridine and 0.5 ml of acetic anhydride and the mixture was heated at 110°C during 3 h with magnetic stirring to give the 1,3,4- thiadiazoline derivative which is filtered and purified by flash chromatography (Figure 2).

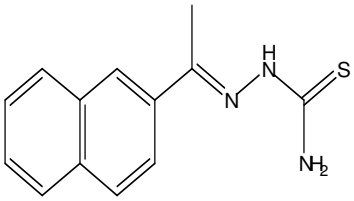
Antitrypanosomal activity (LILIT, Alamar Blue^{TM})

The test is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the "Lilit Alamar Blue" method (Baltz et al., 1985; Hirumi et al., 1994; Räz et al., 1997). The stock solutions of thiosemicarbazones and 1,3,4-thiadiazolines have been prepared from a standard solution an initial concentration (10 mg/ml in DMSO). The trypanosomes are grown in a medium containing 10% of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to 5×10^{-4} tryp/ml. In each well, 50 µL of different dilutions of the stock solution were added to 50 µL of suspension of trypanosomes. The plates were then incubated at 37°C for 72 h in an atmosphere with 5% CO_2. Then, 10 µL of dye "Alamar Blue^{TM}" is added to each well and incubated for 4 h. The dye "Alamar Blue^{TM}" is a reagent for detecting enzymatic activity. The wells in which the concentration of compound is insufficient to inhibit the proliferation of trypanosomes are stained. The CMI is the concentration of unstained wells in which there is the lowest amount of thiosemicarbazone or

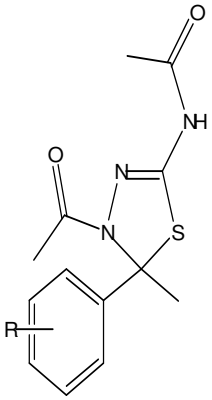
Table 1. Structure and trypanocidal activity of thiosemicarbazones and 1,3,4- thiadiazolines.



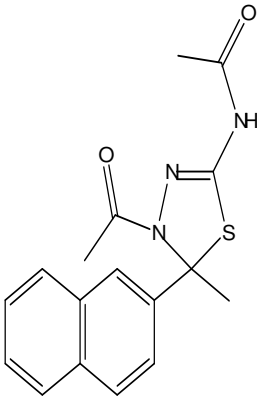
1a-5a



6a



1b-5b



6b

Compounds	R	IC_{50}-moy ± sd (µg/mL)	IC_{50}-moy ± sd (µM)
1a	-	40.95 ± 3.94	212.15 ± 14.22
1b	-	>100	-
2a	2'-Cl	45.40 ± 0.19	199.97 ± 0.56
2b	2'-Cl	53.73 ± 3.1	172.76 ± 8.44
3a	4'-Cl	2.52 ± 0.52	11.07 ± 1.67
3b	4'-Cl	54.72 ± 0.6	175.93 ± 1.83
4a	3'-Br	19.16 ± 0.07	70.44 ± 0.22
4b	3'-Br	>100	-
5a	4'-Br	4.63 ± 1.87	17.02 ± 6.29
5b	4'-Br	47.96 ± 4.94	134.7 ± 16.63
6a	-	2.34 ± 0.65	9.62 ± 2.21
6b	-	16.04 ± 0.09	49.03 ± 6.25

1,3,4-thiadiazole. The plate reading is made in comparison with control wells on a fluorescence plate reader using an excitation wavelength of 530 nm and an emission wavelength of 590 nm.

RESULTS

Six thiosemicarbazones and their corresponding 1,3,4-thiadiazolines were synthesized with yields going from 40 to 89% for the thiosemicarbazone and 27 to 78% for 1'3'4-thiadiazolines. The physical and spectrometric data of the 12 compounds are reported in Table 2. Thin layer chromatography (TLC) shows that thiosemicarbazones with Rf ranging from 0.53 to 0.86 in hydrophobic mobile phases are generally more lipophilic than their corresponding 1,3,4-thiadiazolines, which have Rf up 0.53 to 0.67. The spectrometric data of this table are in conformity with the structures suggested for the products. Thus the IR spectra of the thiosemicarbazones and 1,3,4-thiadiazolines show bands in the range of 3455-3139 cm^{-1} due to the stretching vibration of NH in both types of compounds. The thiosemicarbazones C=N stretching band which corresponds between the thiosemicarbazide part and carbonyl part of the molecule, appears at 1588 or 1587 cm^{-1}. In the ^{1}H NMR spectra the most deshielded proton, which is linked to the central nitrogen atom appears as a broadened singlet between 8.9 and 11.69 ppm for both types of molecules. In the ^{13}C NMR spectra, the thiosemicarbazones C=N bond is indicated by chemical shifts between 145 and 149 ppm while the chemical shift of the C=S bond appears between 176 and 180 ppm, the C=S bond corresponding to the chemical shift between 176 and 180 ppm. Ring closure in 1,3,4-thiadiazolines may be observed by (1) the disappearance of the signal between 176 and 180 corresponding to the thiocarbonyl group, (2) the appearance of a signal between 77 and 81 ppm assigned to C-2 and (3) the signals of the carbonyl and methyl moieties of the acetyl groups incorporated to the molecule. In mass spectrometry, the $[MH]^{+}$ peaks obtained in APCI mode correspond to molecular weights expected for all products. In LC mode, all 1,3,4- thiadiazoles have a single peak confirming their purity. The synthesized compounds were tested for their trypanocidal activity on *Trypanosoma brucei brucei*. The test results are reported in Table 1.

Table 1 shows that thiosemicarbazones **3a**, **5a** and **6a** have the most interesting trypanocidal activity with

Table 2. Compounds physical and spectrometric data.

Acetophenone thiosemicarbazone (1a):

Yield: 85%.**M.p**: 120 to 121°C. **Rf** CH_2Cl_2/MeOH (9/1): 0.53 **MS**: $[M+H]^+$cal 194.07 $[M+H]^+$found 194.04. **IR** data (KBr cm^{-1}): 3408 v(NH), 1587 v(C=N). **^{1}H NMR** data ($CDCl_3$ ppm): 2.3 (3H, s, CH_3) ; 6.5 (2H, s, NH_2) ; 7.2-7.7 (5H, several signals, ArH); 8.9 (1H, s, NH). **^{13}C NMR** data ($CDCl_3$ ppm) 12.28 (CH_3); 124-135 (aromatic C); 146 (C=N); 177 (C=S).

2'-chloroacetophenone thiosemicarbazone (5a):

Yield: 40%. **M.p** : 180-181°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.53 **MS**: $[M+H]^+$cal 228.03 $[M+H]^+$found 228.05. **IR** data (KBr cm^{-1}): 3400 v(NH), 1587 v(C=N). **^{1}H NMR** data (DMSO ppm): 2.28 (3H, s, CH_3); 7.38-7.49 (4H, several signals, ArH); 7.62 (1H , br s, NH_2); 8.24 (1H , br s, NH_2); 10.34 (1H, s, NH). **^{13}C NMR** data (DMSO ppm) 18.46 (CH_3); 127-138 (aromatic C); 148.84 (C=N); 179.21(C=S).

4'-chloroacetophenone thiosemicarbazone (5a):

Yield: 70%. **M.p** : 194 to 196°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.80. **IR** data (KBr cm^{-1}): 3418, 3139 v(NH), 1587 v(C=N). **^{1}H NMR** data (DMSO-d_6 ppm) 2.29 (3H, s, CH_3); 7.41-7.96 (4H, several signals, ArH); 8.32 (2H, s, NH_2); 10.26 (1H, s, NH). **^{13}C NMR** data ($CDCl_3$ ppm): 14.12 (CH_3); 128.42-136.74 (aromatic C); 146.80 (C=N); 179.25 (C=S).

3'-bromoacetophenone thiosemicarbazone (4a):

Yield: 75%. **M.p**: 172-173°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.86. **IR** data (KBr cm^{-1}): 3392, 3144 v(NH), 1588 v(C=N). **^{1}H NMR** data (DMSO-d_6 ppm) 2.28 (3H, s, CH_3); 7.34-7.98 (4H, several signals, ArH); 8.19 (2H, s, NH_2); 10.25 (1H, s, NH). **^{13}C NMR** data ($CDCl_3$ ppm): 13.93 (CH_3); 122.01-140.01 (aromatic C); 146.27 (C=N); 179.01(C=S).

4'-bromoacetophenone thiosemicarbazone (5a):

Yield: 83%. **M.p** : 198-199°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.86. **IR** data (KBr cm^{-1}): 3410, 3193 v(NH), 1587 v(C=N). **^{1}H NMR** data (DMSO-d_6 ppm) 2.28 (3H, s, CH_3); 7.54-7.88 (4H, several signals, ArH); 8.00 (1H, s, NH_2); 8.38 (1H, s, NH_2); 10.27 (1H, s, NH). **^{13}C NMR** data (DMSO-d_6 ppm) 13.79 (CH_3); 122.68-136.83 (Aromatic C); 146.59 (C=N); 178.96 (C=S).

Acetonaphtone thiosemicarbazone (6a):

Yield: 89%. **M.p**: 180-181°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.86. **MS**: $[M+H]^+$cal 244.09 $[M+H]^+$found 244.04. **IR** data (KBr cm^{-1}): 3435, 3193 v(NH), 1606 v(C=N). **^{1}H NMR** data (DMSO-d_6 ppm) 2.4 (3H, s, CH_3); 7.54-7.88 (4H, several signals, ArH); 8.00 (1H, s, NH_2); 8.38 (1H, s, NH_2); 10.3 (1H, s, NH). **^{13}C NMR** data (DMSO-d_6 ppm) 11.89 (CH_3); 122.18-133.19 (aromatic C); 145.71 (C=N); 177.05 (C=S).

5-Acetamido-3-*N*-acetyl-2-methyl-2-phenyl-1,3,4-thiadiazoline (1b):

Yield: 57%. **M.p** : 224-225°C. **Rf** CH_2Cl_2/MeOH (9/1): 0.53. : **MS**: $[MH]^+$cal 278.09564 $[MH]^+$found 278.09577. **IR** data (KBr cm^{-1}): 3145 v(NH), 1694, 1631, 1615 v(C=O amide). **^{1}H NMR** data ($CDCl_3$ ppm) 1.84 (3H, s, CH3); 2.22 (3H, s, CH_3 amide); 2.29 (3H, s, CH_3 amide); 7.15-7.35 (5H, several signals, ArH); 9.14(1H, s, NH). **^{13}C NMR** data ($CDCl_3$ *ppm)* 22.87 (CH_3); 25.89 et 26.86 (CH_3 amide); 80.03 (C_2 in the ring); 124.99-142.82 (aromatic C); 143.48 (C=N); 168.84 and 169.27 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(2´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (2b):

Yield: 27%. **M.p** : 215-217°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.58 **MS**: $[MH]^+$cal 312.0568 $[MH]^+$found 312.0565. **IR** data (KBr cm^{-1}): 3160 v(NH), 1698, 1644, 1611 v(C=O amide). **^{1}H NMR** data ($CDCl_3$ ppm) 1.86 (3H, s, CH_3); 2.26 (3H, s, CH_3 amide); 2.36 (3H, s, CH_3 amide); 7.19-7.42 (4H, several signals, ArH); 9.61 (1H, s, NH). **^{13}C NMR** data ($CDCl_3$ ppm) 22.96 (CH_3 amide); 23.01 (CH_3 amide); 28.85 (CH_3); 78.35 (C_2 in the ring); 126.64-137.27 (aromatic C); 144.37 (C=N); 168.68 et 168.78 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(4´-chlorophenyl)-2-methyl-1,3,4-thiadiazoline (3b):

Yield: 58%. **M.p**: 214-216°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.61 **MS**: $[MH]^+$cal 312.0568 $[MH]^+$found 312.0567 **IR** data (KBr cm^{-1}): 3146 v(NH), 1694, 1633, 1617 v(C=O amide). **^{1}H NMR** data ($CDCl_3$ ppm) 1.75 (3H, s, CH_3); 2.22 (3H, s, CH_3 amide); 2.24 (3H, s, CH_3 amide); 7.19-7.27 (4H, several signals, ArH); 10.14 (1H,s,NH). **^{13}C NMR** data ($CDCl_3$ ppm) 22,49 (CH_3); 23.78 (CH_3 amide); 26.62 (CH_3 amide); 78.90 (C2 in the ring); 126.68-141.31 (aromatic C); 144.86 (C=N); 169.40 et 169.56 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-(3´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (4b):

Yield: 76%. **M.p** : 238-239°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.57 **MS**: $[MH]^+$cal 358.0048 $[MH]^+$found 358.0046. **IR** data (KBr cm^{-1}): 3148 v(NH), 1695, 1614 v(C=O amide). **^{1}H NMR** (DMSO ppm) 2,03 (3H, s, CH_3); 2.20 (3H, s, CH_3 amide); 2.27 (3H, s, CH_3 amide); 7.26-7.52 (4H, several signals, ArH); 11.69 (1H, s, NH). **^{13}C NMR** data (DMSO ppm) 22,40 (CH_3); 23,58 (CH_3 amide); 26,30 (CH_3 amide); 77,86 (C2 in the ring); 121,72-142,30 (aromatic C); 144.36 (C=N); 167,77 et 169,45 (C=O amide).

Table 2. Contd.

5-Acetamido-3-*N*-acetyl-2-(4´-bromophenyl)-2-methyl-1,3,4-thiadiazoline (5b):

Yield: 78%. **M.p** : 211-213°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.67 **MS**: $[MH]^+$cal 358.0048 $[MH]^+$found 358.0038. **IR** data (KBr cm^{-1}): 3218, 3148 v(NH), 1693, 1614 v(C=O amide). **^{1}H NMR** data ($CDCl_3$ ppm) 1.75 (3H, s, CH_3); 2.22 (3H, s, CH_3 amide); 2.24 (3H, s, CH_3 amide);7.19-7.24 (4H, several signals, ArH); 10.32 (1H, s, NH). **^{13}C NMR**data ($CDCl_3$ *ppm)* 22.54 (CH_3); 23.85 (CH_3 amide); 26.59 (CH_3 amide); 78.85 (C2 in the ring); 121.93-141.85 (aromatic C); 144.34 (C=N); 169.39 and 169.55 (C=O amide).

5-Acetamido-3-*N*-acetyl-2-methyl-2-naphtyl-1,3,4-thiadiazoline (6b):

Yield: 67%. **M.p**: 188-190°C. **Rf** CH_2Cl_2/AcOET (2/1): 0.61 **MS**: $[MH]^+$cal 328.1114 $[MH]^+$found 328.1112. **IR** data (KBr cm^{-1}): 3183 v(NH), 1700, 1642, 1616 v(C=O amide). **^{1}H NMR** data ($CDCl_3$ ppm) 1.40 (3H, s, CH_3); 2.37 (3H, s, CH_3 amide); 2.42 (3H, s, CH_3 amide); 7.38-7.79 (7H, several signals, ArH); 9.61 (1H, s, NH). **^{13}C NMR** data ($CDCl_3$ ppm) 22.30 (CH_3); 24.14 (CH3 amide); 26.86 (CH_3 amide); 79.82 (C2 in the ring); 123.86-139.80 (aromatic C); 144.37 (C=N); 168.68 and 168.78 (C=O amide).

respective IC_{50} values of 11.07, 17.02 and 9.62 µM. Compound 6a appears as the most trypanocidal of all tested products. However the 1,3,4-thiadiazoline 6b have a moderate trypanocidal activity with IC_{50} value of 49.03 µM.

DISCUSSION

These thiosemicarbazones are likely to inhibit cysteine proteases such as cathepsins: TbcatB and rhodesain. It has been suggested that these proteases may be involved in nutrient acquisition, degradation of host proteins, evasion of the host immune response, or crossing of the blood brain barrier and are essential for the parasite survival (Caffrey et al., 2001; Mackey et al., 2004). The inhibition of these proteases by thiosemicarbazones would consequently result to the death of the parasite *Trypanosoma brucei brucei*. Substitution on the aromatic ring influence trypanocidal activity. Thus, compound **1a**, which has no substituent on the nucleus, has the highest IC_{50} value (IC_{50} = 212.15 µM). Substitutions at position 2 and 3 respectively with chlorine (**2a** IC_{50} = 199.97 µM) and bromine (IC_{50} = 70.44 µM **4a**) are less interesting than substitution at position 4 by chlorine (**3a** IC_{50} = 11. 07 µM) or bromine (**5a** IC_{50} = 17.02 µM). In this case, the chlorine compound is more active than the bromine compound. The substitution of phenyl with naphthyl in the thiosemicarbazone **6a** (IC_{50} = 9.62 µM) increases the trypanocidal activity. This compound is the most active of all tested compounds. Its IC_{50} value is in the same range (IC_{50} < 10 µM) with these of thiosemicarbazones of (3,5-dichlorophenyl)(p-tolyl) methanone (IC_{50} = 1.1 µM) , 1-(3,5-dichlorophenyl)-3-phenylpropan-1-one (IC_{50} = 1.3 µM), 1-(3-chlorophenyl)-3-phenylpropan-1-one (IC_{50} = 3.7 µM), 1-(3-fluorophenyl)-3-phenylpropan-1-one (IC_{50} = 6 µM)…, synthesized by Mallari et al and tested on Trypanosoma brucei brucei in culture (Mallari et al., 2008). According to the work of Du et al. (2002) thiosemicarbazones are trypanocidal when their IC_{50} values are less than 10 µM, moderate trypanocidal if these values are between 10 and 100 µM, and have little or no activity when their IC_{50} are higher than 100 µM. As for the structure-activity relationship, the most active thiosemicarbazone **6a** leads to the most active thiadiazoline **6b**. Thus, the thiadiazoline **6b** have a moderate trypanocidal activity. This activity is higher than all the other thiadiazolines and the thiosemicarbazones **1a**, **2a** and **4a** with an IC_{50} value of 49.03 µM.

However, the 4-brominated thiosemicarbazone **5b** is more active than the 4-chlorinated compound **3b**. The thiadiazolines **1b**, **2b**, **3b**, **4b** and **5b,** with a weak activity, exhibit IC_{50} values greater than those of their corresponding thiosemicarbazones.

This study can conclude that the thiosemicarbazones have higher trypanocidal activity than the one of the corresponding thiadiazolines. However some thiadiazolines can have interesting trypanocidal activities and even higher than other thiosemicarbazones.

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